Dr. Anand Ganesan

Task in Litigation

- Evaluate the biologically plausible mechanism of action by which the PDE5 inhibitor tadalafil causes melanoma progression.

Summary of Opinions

- It is my opinion to a reasonable degree of scientific and medical certainty that:
  - There is a plausible biological mechanism by which PDE5 inhibitors, including tadalafil, promote increased growth and invasiveness of melanoma – including causing a dormant undetectable melanoma to become a diagnosable disease
  - The Arozarena and Dhayade publications demonstrate how this mechanism functions and that this mechanism is generalizable to most, if not all melanomas
  - The therapeutic dose and duration is sufficient to cause melanoma growth and invasion
  - This mechanism is further supported by the consistent and significant association found between PDE5 inhibitors and melanoma in the epidemiological literature

Methodology

- Conducted extensive literature search
- Reviewed the epidemiologic data demonstrating an association between PDE5 inhibitor use and melanoma
- Examined studies related to the mechanism of how PDE5 promotes melanoma progression
  - The mechanism of action of a PDE5 inhibitor
  - PDE5 presence in melanocytes and melanoma cells
  - In vitro and in vivo studies demonstrating PDE5 inhibition effect of growth and invasion
  - Evaluation of signaling pathways involved

Methodology

- Examined evidence related to PDE5 inhibitor use and potential anti-cancer effects
  - Considered whether studies were conducted in melanoma or other cancers
  - Considered which stage of cancer studies evaluated
  - Considered which agents were used (specific PDE5 inhibitors versus cGMP analogues or non-specific PDE inhibitors)
  - Considered the context of the mouse models (pauci inflammatory, chronic immune microenvironment)
  - Considered the effect of PDE5 inhibitors on the immune system and relevance to melanoma
- Reviewed internal documents produced by Eli Lilly, including preclinical studies and employee deposition testimony

Definitions of Terms in Cancer Biology

Proliferation- the process that results in the increased number of cells as a result of cell division.

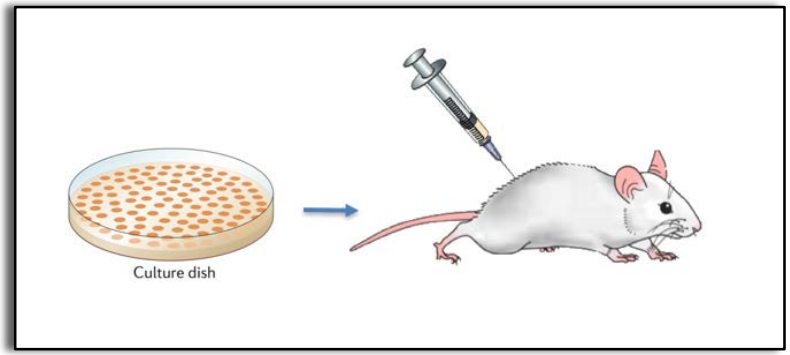
Growth- the process of increasing physical size.

Tumor Volume- the size of a cancer as measured by the amount of space taken up by a tumor.

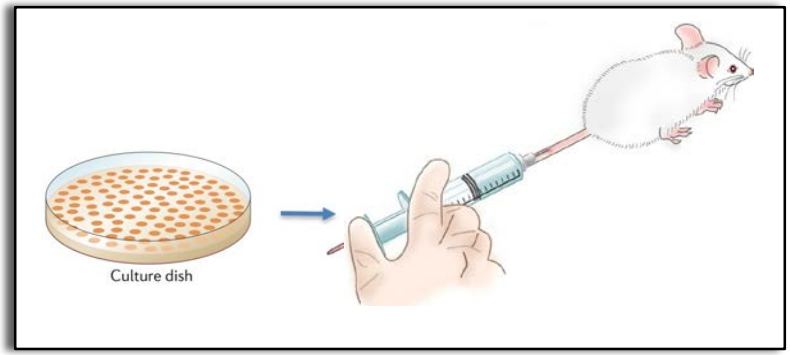
Invasion- The direct penetration of cancer cells into adjacent tissues. For melanoma, this is the first step in progression. Physically, this refers to penetration through an adjacent tissue barrier, in this case the dermis, which contains collagen.

Metastasis- tumor cells break away from where the tumor was first formed, enter the blood stream, penetrate a new tissue, and survive in the new tissue.

Models to Look at Tumor Growth



- Culture cells in a dish
- Put in a fluorescent marker
- Inject the cells in the flank of a mouse without a functional immune system
- Look at the movement of the tumor cells within the tumor itself
- Models tumor growth and invasion



- Culture cells in a dish
- Put in a fluorescent marker to label the cells
- Inject the cells into the tail vein of a mouse
- Looks at the movement of the tumor cells from the bloodstream to the lung
- Models extravasation of tumor cells out of the bloodstream- only late stages of metastasis

Dose Considerations

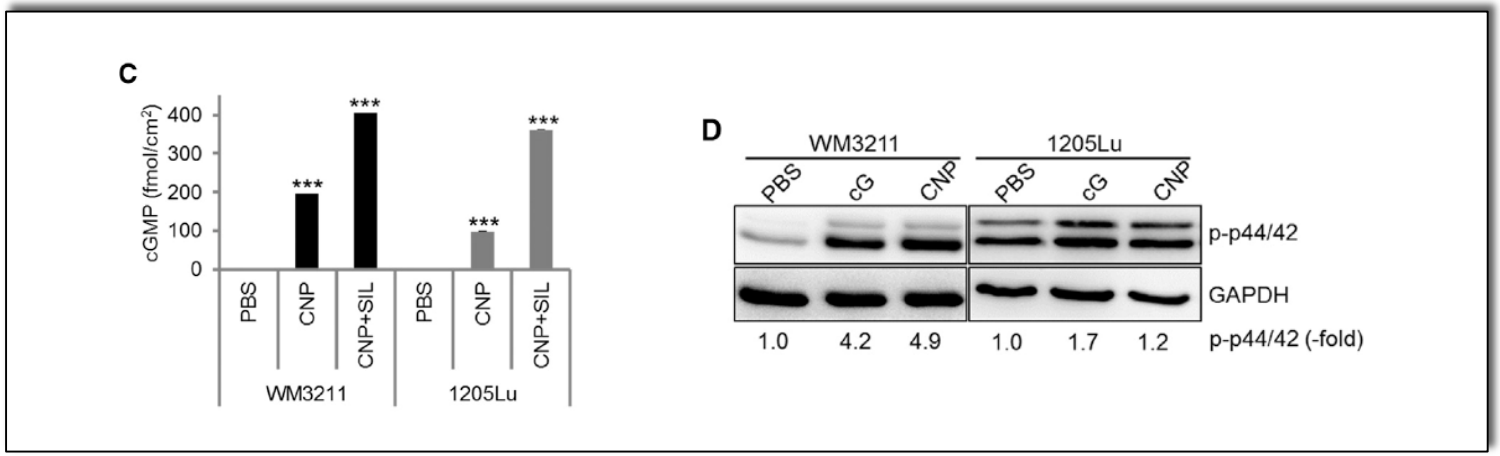
- Bioavailability
  - The bioavailability of the drug in mice is 17% compared to 41% in humans.
- Drug excretion
  - Sildenafil is excreted more than 2.4 times as fast in mice as in humans.

Dhayade 2016 – Figure 7

"Human Melanoma Cells Also Express a Growth-Promoting cGMP Pathway"

- In two human melanoma cell lines, administration of sildenafil to cells causes increase in MAPK activation and also increase in cGMP levels within 10 minutes
- This series of experiments led the authors to conclude that "human melanoma cells can express a growth-promoting cGMP pathway that is also responsive to pharmacological stimulation with sildenafil." Dhayade et al., p. 5.

Figure 7 C and D



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Dhayade 2016 –Figure S5

"Model of cGMP Signaling in Melanoma Pathogenesis"

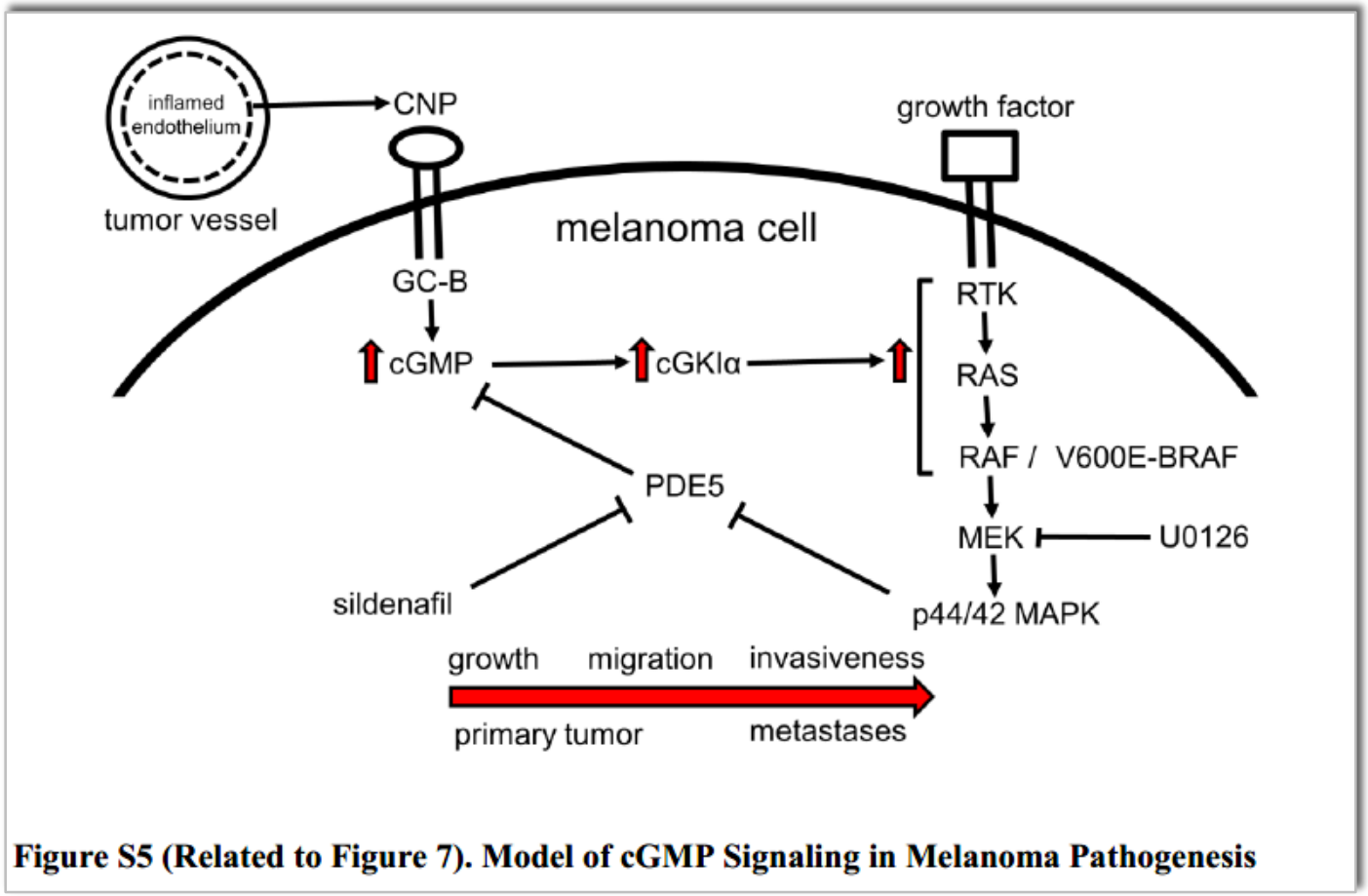

Figure S5 (Related to Figure 7). Model of cGMP Signaling in Melanoma Pathogenesis

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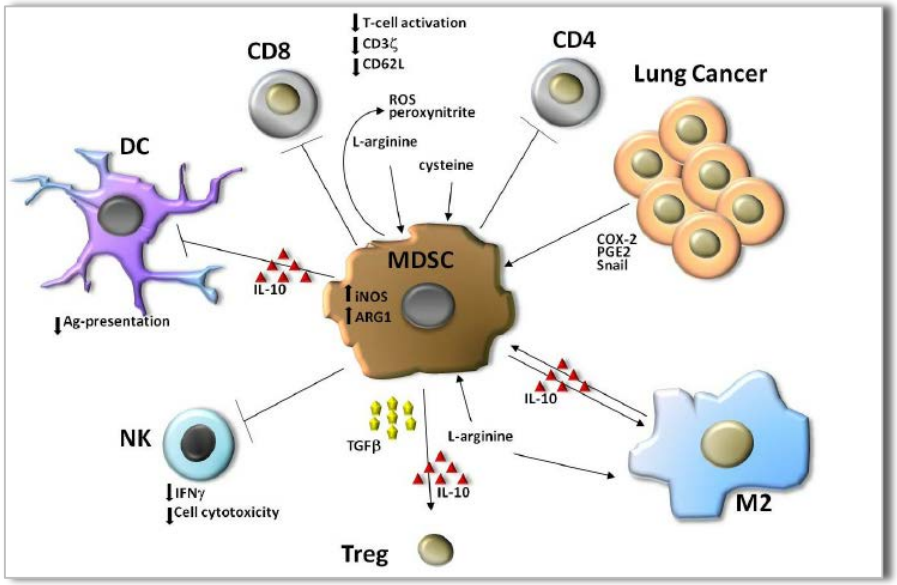
Additional Literature Regarding PDE5 Inhibitors and Cancer

- Direct effect studies
  - These are the studies that test whether a PDE inhibitor has a direct effect on a cancer cell.
- cGMP analog studies.
  - These studies look at the effect of cGMP analogs, which are experimental tools, on cancer cells. They do not involve PDE5 inhibitors.
- Immunotherapy studies.
  - These studies involve PDE5 inhibitors being used in conjunction with other cancer treatment drugs and evaluating the effects on the immune system.

MDSCs in the Immune Microenvironment

- "MDSCs have been reported to be enriched during the tumor progression and to display a strong inhibition of antitumor T cells immune responses by multiple mechanisms" Hassel et al. at 1.
- Hassel et al. includes a reference to Jordan KR 2013 "Myeloid derived suppressor cells are associated with disease progression and decreased overall survival in advanced-stage melanoma patients." MDSCs are not seen in early stage melanoma in this paper.



Srivastava et al 2012 Lung Tumor Microenvironment and myelomonocytic cells.

Note MDSCs are activated by established tumors (lung cancer in this example)

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Overall Opinion on Additional Literature Regarding PDE5 Inhibitors and Cancer

- The direct anti-cancer publications do not demonstrate any anti-cancer effect of a selective and potent PDE5 inhibitor on early stage melanomas
- The cGMP analog studies are done with less specific experimental tools, not PDE5 inhibitors and were not testing the effect of PDE5 inhibition on melanoma cells.
- The publications regarding immunotherapy, which I considered more relevant and discussed in my report, use of PDE5 inhibitors are limited in application to late stage melanomas or those that have progressed which have more MDSCs, not early stage tumors. They are also not proposed as a monotherapy, but only for use in conjunction with cancer treatments.

Conclusion

- It is my opinion to a reasonable degree of scientific and medical certainty that:
  - There is a plausible biological mechanism of action by which PDE5 inhibitors can cause increased growth and invasiveness in melanoma, including causing a dormant undetectable melanoma to become a diagnosable disease.
  - Arozarena and Dhayade demonstrate that PDE5 inhibitors promote melanoma invasiveness and growth.
  - The therapeutic dose and duration is sufficient to cause melanoma growth and invasion.
  - There is sufficient evidence in the epidemiologic literature that PDE5 inhibitor use is associated with increased risk of developing melanoma.
  - There is evidence that PDE5 inhibitors affect MDSC function, which may impact the growth of some advanced melanomas, but does not negate the effect of melanoma progression seen in Arozarena and Dhayade.